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Qualitative and quantitative risk assessment

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Abstract

Risk assessment and HACCP are related, but fundamentally different processes. Four major elements of risk assessment are commonly described (hazard identification; exposure assessment; dose-response assessment or hazard characterization; and risk characterization). Some similarities exist between the inputs for the first elements of risk assessment (hazard identification) and HACCP (hazard analysis). However, HACCP involves the identification of critical control points of a process for the purpose of producing a 'safe' product, and thus is essentially a risk management procedure that does not estimate risk with attendant uncertainty as in the formal structured procedure described for risk assessment. For quantitative models in microbial risk assessment, exposure assessment requires data for pathogen occurrence, density or level, and distribution in foods and live animals, parameters for growth and decline, and consumption information. A crucial difference between chemical and microbial risk assessment is that for the latter, exposure models must account for pathogen growth and deactivation, termed predictive microbiology. This field has emphasized prediction of the expected changes in a population of organisms and is extended by an example accounting for the stochastic or random variability of microbial growth in a given circumstance. Dose-response assessment, the third element of risk assessment, is the crucial link between exposure in food to adverse human health outcomes. Data, from controlled human studies with healthy adult volunteers to describe dose-response relationships, are limited. Differences between human sub-populations may be inferred from animal studies, based on a common mechanism, such as the observed pre-disposition of antibiotic-treated animals to subsequent challenges with enteric pathogens. Professional organizations, such as the Society for Risk Analysis, can greatly assist governments, industry, academia, and stakeholders in scrutinizing the risk analysis processes of risk assessment, risk management, and risk communication. Published by Elsevier Science Ltd.

Keywords: Stochastic process; Susceptible sub-populations; Mechanisms of pathogenecity

1. Introduction

Risk assessment and HACCP are related, but fundamentally different processes. Risk assessment is a structured science-based process to estimate the likelihood and severity of risk with attendant uncertainty. For risk assessment many organizations recognize four major elements: hazard identification; exposure assessment; dose-response assessment or hazard characterization; and risk characterization (Fig. 1). A risk analysis links a risk assessment with both risk communication and risk management. The starting point of a risk analysis, however, need not be a risk assessment. Rather, in the US, risk communication is receiving increasing attention as the starting point for risk analyses for transmissible spongiform encephalopathies, *Salmonella enteritidis*, and *E. coli* O157:H7 (USDA, www.fsis.usda.gov/OPHS). Dialogue with stakeholders, including industry, academia, government, and the general public, is increasingly viewed as essential throughout the entire risk analysis process. The results of risk assessments are weighed by risk managers with other factors to support decision-making processes.

HACCP systems are examples of risk management programs. Specifically, HACCP involves the identification of critical control points of processing, and processing parameters for these that if met would assure that the produced product meets specified standards that imply a safe product. Some similarities exist between the inputs for the first elements of risk assessment (hazard identification) and an hazard analysis (of a HACCP program). For example, hazard identification and hazard analysis might both consider data from epidemiological investigations: risk factors, food vehicles, associations with adverse health outcomes, the nature

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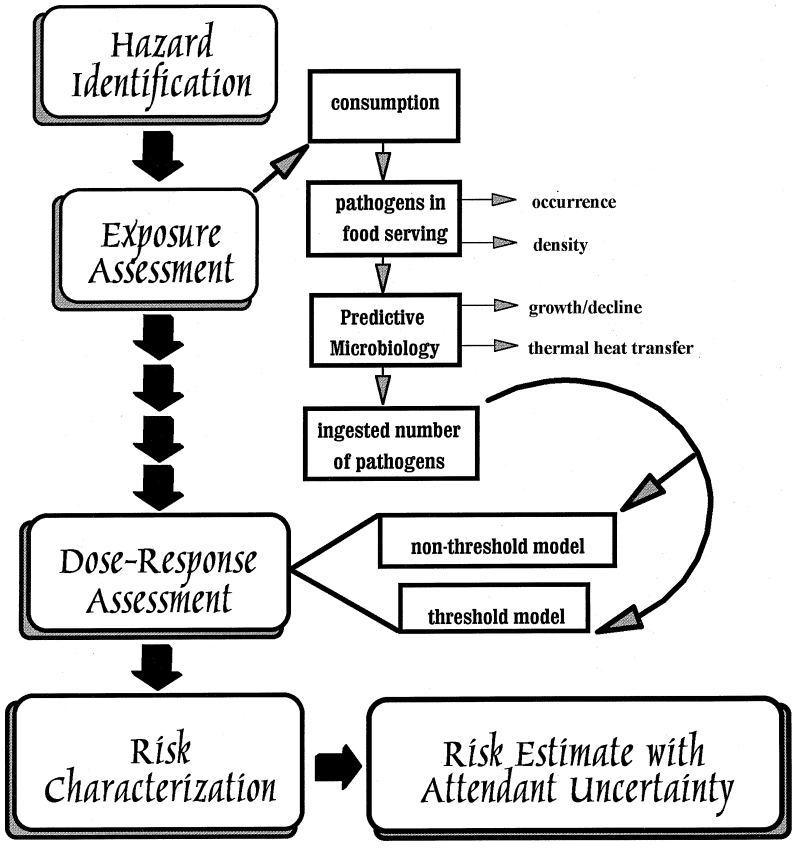

Fig. 1. A framework for microbial risk assessment (Marks, 1998, with permission from *Risk Analysis*).

and severity of illness, and effects in sensitive sub-populations. Together with a cost/benefit analyses, the results of the risk assessment can be used for defining acceptable product characteristics or processing goals for a HACCP program.

Qualitative hazard identification or risk assessment can assist a risk manager in priority setting, policy decision-making, such as decisions to allocate resources to sampling. Some qualitative assessments can contribute to the published literature on risk analysis and serve as tools to identify and prioritize research needs. The Codex Committee on Food Hygiene is committed to valuing qualitative information for risk assessment as well as encouraging the use of quantitative data to the extent possible (CCFH, 1998).

Many factors may influence the decision to conduct a qualitative versus a quantitative risk assessment. Obviously, if no data are available to make inferences from, then a quantitative risk assessment would not be possible. Constraints in data quality, time, personnel, or resources may not permit a full quantitative risk assessment. However, data gaps are not necessarily a barrier to quantitative risk assessment. Our bias has been towards 'Letting the data speak!', using thorough data analysis, formal inferencing, and striving for complete documentation of variability and uncertainty (Marks, 1998a,b; Coleman, 1998). The use of experts for a risk assessment can be consistent with this approach. Kaplan (1992) advocates eliciting the *data* from the experts, not their opinions of the possible values of parameters. Good practice for risk assessors would include elicitation of the *evidence* from the experts and creation of a consensus state-of-knowledge curve as a means to address data gaps so that uncertainty and variability can be computed (Kaplan, 1992). Otherwise, the judgements and opinions of experts may impose on the risk assessment significant bias and overconfidence that could misinform decision makers about the magnitude of risk and attendant uncertainty.

For quantitative models, food safety systems are complex to model, both biologically and mathematically. Although models are admittedly simplifications of reality, realistic and plausible models for farm-to-table risk assessment are not simple. For example, Fig. 1 illustrates some difficulties in simply modeling microbial hazards. The figure depicts the need to realistically model microbial growth and decline, and cooking processes using thermal heat transfer equations. Examination of the data with a mechanistic perspective might be useful for developing simple approximations for the complex analytical model systems. Because of the complexity of the model and the desire on the part of

risk assessors to present simple and succinct models, particular attention is needed to assure that the risk assessment process is transparent, which enables critical review of the work.

2. Exposure assessment

Exposure assessment models requires data for pathogen occurrence, distribution of densities or levels in food, parameters for growth and decline, and consumption information (Marks, 1998a). Of course, the availability of high quality farm-to-table exposure data are limited. When data are available, risk assessors must account for measurement and sampling errors for microbiological methods, such as the Most Probable Number (MPN) methods, which can produce false negative results (Fontaine, 1978; Marks, 1998b). A crucial difference between chemical and microbial risk assessment is that for the latter, exposure models must account for pathogen growth and deactivation. Modeling growth and deactivation is termed predictive microbiology (McMeekin, 1993). Predictive microbiologists have generally developed deterministic models that estimate the expected value, or average growth rate, often based on growth of cocktails of microbial strains in broth cultures without the competitive microflora associated with specific food substrates (USDA/ARS, 1999). Thus, information concerning differences of growth rates and lag times for different pathogenic strains is often unknown. Some predictive models have limited independent validation and thus limited plausibility for risk assessment. Growth rates and lag times may differ between pathogenic strains by orders of magnitude (Whiting, 1998, personal communication).

Microbial growth is not an all-or-none, deterministic process, but rather a stochastic or random process involving a population cells (Edelstein-Keshet, 1988; Karlin, 1971; Keen, 1992; Kendall, 1948). Stochastic variation of microbial dynamics is critically important for use in quantitative risk assessments to describe the full range of possible risk. Development of methods to account for variations of the natural range of population growth and decline are needed to support public health risk assessment.

One elementary but important example of stochastic process in exposure assessment is simple linear birth process described in the field of population biology. The differential equation $dN(t)/dt = \mu N(t)$, where t represents time, describes the rate of change of the number of organisms, $N(t)$, in the population, to be proportional to $N(t)$, with proportionality constant, μ. This constant, μ, is often referred to as the specific or exponential growth rate. For example, the above equation predicts that at time t_1, a population of 10 cells increasing by a factor of 10 would be expected to increase by 90 cells so that the final population size would be 100 cells. However, a more realistic solution can be mathematically derived under suitable assumptions. One assumption is a simple linear birth process in which the probability of a cell division in a small increment of time is a constant, μ, and the possibility of two cells dividing in a small increment of time is very small. Then it can be derived that the distribution of the increase number of cells at time t is that of a negative binomial distribution with probability parameter $e^{-\mu t}$ and number parameter N_0, the initial number of cells. The expected increase in the number of cells is $N_0(e^{\mu t} - 1)$. In the example given, $N_0 = 10$, and $\mu = \ln(10)/t_1$. Using the negative binomial distribution, the actual increase might range from 30 to 185 cells with 99% probability. Thus, the negative binomial distribution predicts a fivefold range of relative growth due solely to the inherent variability of microbial population growth.

A second simple example of stochastic growth process in exposure assessment extends the model of the above paragraph by assuming the existence of a lag phase, a period of time before a cell can divide. The mathematics to describe the exact distribution of growth with lag is complex. Cells prior to time 0 are assumed to be in a stationary state. After a change in the environment that causes growth, time, t_0, is needed for an organism to acclimate itself and to start the machinery necessary for growth. Once the time exceeds t_0, the cell divides and the new cells grow and multiply. To develop a model for the number of cells in the above described random model, it is convenient to keep track of the set of original cells, O, and the set of new cells, D, that are a result of cell division. As an example, the D cells are assumed to follow the simple linear birth process described above with parameter μ. A new variable must be introduced, which describes the random, lag phase variable, defined as $\lambda(t)$, the infinitesimal cell 'death' rate of the O cells. In one sense, $-\lambda(t)$ represents the probability that the O cell divides into two D cells and thus no longer exists at time t. Symbolically, this can be represented by $O \rightarrow_{\lambda} D + D$. Further the cells created from a division of D cells are assumed to behave as D cells and divide according to the simple linear birth process described in the previous paragraph.

In this application, the initial population is assumed to consists of one cell, so that $N_0 = 1$. The probability for the single cell surviving at time t is $e^{-\gamma(t)}$, where $\gamma(t) = \int_0^t \lambda(\tau)\,d\tau$. Let t_0 be the time that the cell divides. The cumulative distribution function for the random variable t_0 is $H(t) = \text{Prob}(t_0 < t) = 1 - e^{-\gamma(t)}$, and the probability density function of t_0 is: $h(t) = \gamma'(t)e^{-\gamma(t)} = \lambda(t)e^{-\gamma(t)}$. The probability of n organisms at time, t, that is $\text{prob}(N(t) = n) = p_n(t)$ can be derived and is given in Eq. 1.

$$p_1(t) = e^{-\gamma(t)}$$

$$p_n(t) = (n-1)\int_0^t e^{-2\mu(t-\tau)}(1-e^{-\mu(t-\tau)})^{n-2}\,dH(\tau), \quad n>1. \tag{1}$$

The expected value of $N(t)$ can be calculated (Eq. 2):

$$E(N(t)) = e^{-\gamma(t)} + 2\int_0^t e^{\mu(t-\tau)}\,dH(\tau). \tag{2}$$

The variance can be calculated (Eq. 3):

$$\mathrm{var}(N(T)) = 6\int_0^t e^{2\mu(t-\tau)}\,dH(\tau) - 4\int_0^t e^{\mu(t-\tau)}\,dH(\tau) + E(N(t))(1-E(N(t))). \tag{3}$$

A convenient probability distribution for the time of cell division with density of form $\gamma'(t)e^{-\gamma(t)}$ is the Weibull distribution, for example, $=\gamma(t)=t^a/b$, where a and b are constants. Thus the above model has three parameters: a, b, and μ. Fig. 2 provides an example of the above 2-stage birth model assuming initially a single cell with $a=5$, $b=10$, and $\mu=1$. By assumption, the number of cells cannot decrease, thus the lognormal distribution is possible to use for approximating the actual percentiles. Curves are plotted for the expected $\log_{10}$ relative growth and estimates of the 95% percentile of the $\log_{10}$ relative growth using the extreme value, lognormal, and the negative binomial distributions with probability parameter $1/E(N(t))$ and number parameter equal to 1. In addition, percentiles determined using 10 000 Monte Carlo simulations computed on @Risk®, version 3.5b, are plotted (Fig. 2). The star bursts on the figure represent the exact calculations of time (performed on Mathcad® version 4.0) when the probability of the number of cells being less than or equal to n equals 95%, for $n=1,2,\ldots 6$, using Eq. 1.

The expected value from Eq. 2 is shown in the lower curve (Fig. 2). This distribution reflects expected relative growth with lag. In contrast, to depict variability, the remaining lines shown as the upper curves in the figure represent the 95th percentiles. In generating the number of organisms for risk assessment, 5% of the generated values would fall above these curves. The lognormal and the extreme value distributions give virtually the same estimates and both provide very good estimates for time >1.5; the approximation using the negative binomial overestimates the actual percentiles as t gets larger. A clear objective of developers of risk assessment methodology is to identify approximations to the exact distributions among the many more convenient distri-

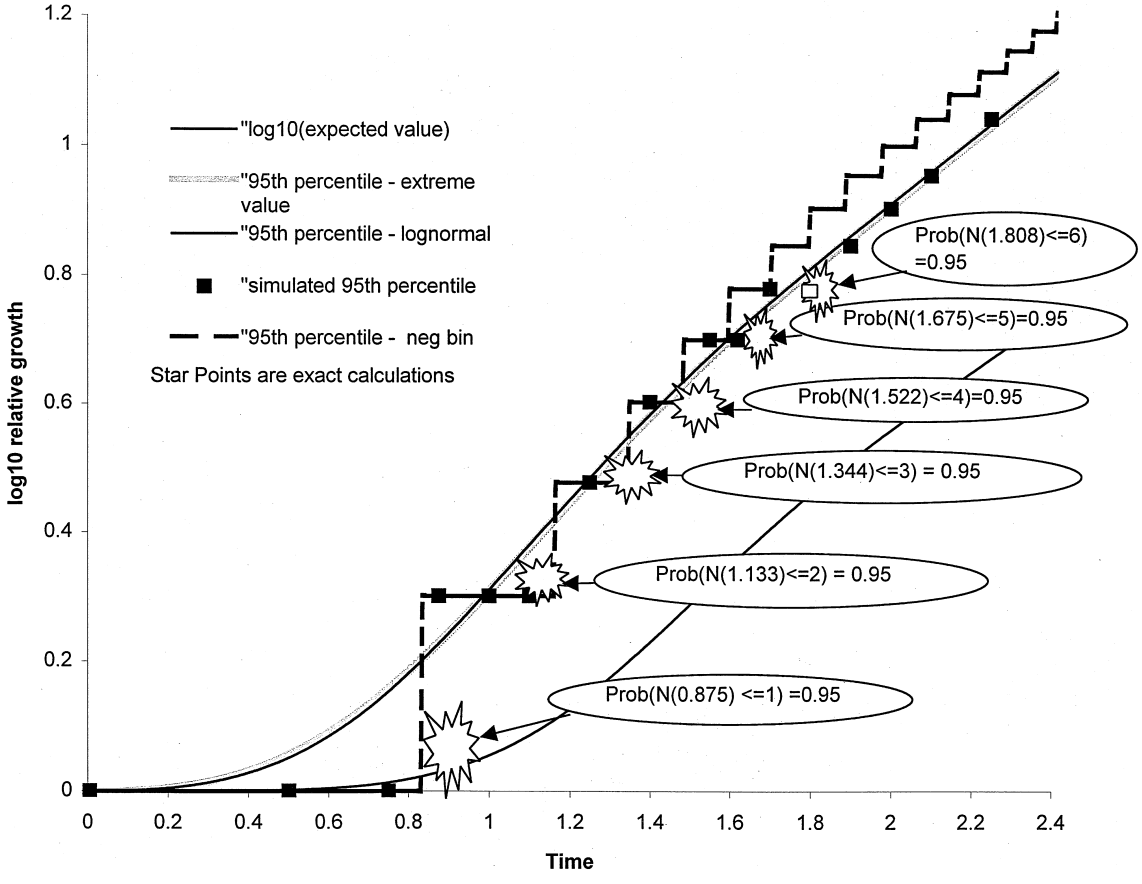

Fig. 2. Exact and approximate distributions for stochastic growth with lag.

butions available in many commercial software products.

3. Dose-response modeling

Dose-response assessment, the third element of risk assessment, is the crucial link between exposure in food and adverse human health outcomes. For dose-response assessment, different mathematical and biological challenges arise. Ideally, risk assessors would like to base their dose-response models on data from representatives of the entire human population in multiple-dose challenge studies with the administration of the pathogen of interest and observation of the likelihood and severity of illness. Data are limited from controlled human studies with healthy volunteers to describe dose-response relationships. A fine review of much of the available human data for microbial dose-response modeling was prepared by Teunis and colleagues (1996). Many of these studies involved healthy male adult volunteers, hardly typical of more sensitive human sub-populations.

An example of a dose-response model based on data from human clinical studies is non-typhoid salmonellosis (Coleman, 1998). Although the taxonomy of the *Salmonella* genus is in flux (D'Aoust, 1997), the *Salmonella* strains administered to healthy human males could be considered the same species, differentiated by surface antigens into serovar classifications Anatum, Bareilly, Derby, Meleagridis, and Newport (McCullough & Eisle, 1951a–c). Data for fours strains of *Salmonella* pullorum (McCullough & Eisle, 1951d) were excluded from the analysis (Coleman, 1998) because these strains appear to function as specific poultry pathogens rather than as human pathogens. The observed human data for the non-typhoid *Salmonella* serovars are labeled as diamond symbols (Fig. 3). Great variability is noted between serovars and strains (Coleman, 1998), some non-pathogenic at high doses in healthy adults, and others that cause 100% of the treatment groups to become ill. Rather than imposing conservatism in the risk assessment model and selecting only the most virulent strains, the non-typhoid salmonellosis model treats the available human data as strains representative of the population of strains present in the diet. This approach permits more realistic modeling in Monte Carlo simulations which select randomly from among the sample of available strains to predict a dose-response relationship for an unknown strain. If these strain differences are not accounted for, then the models represented in Fig. 3 as two possible models, one using the the extreme value (Gompertz) function and the other using the logistic function, are both rejected for poor fit (Coleman, 1998). By incorporating strain variability, through using an

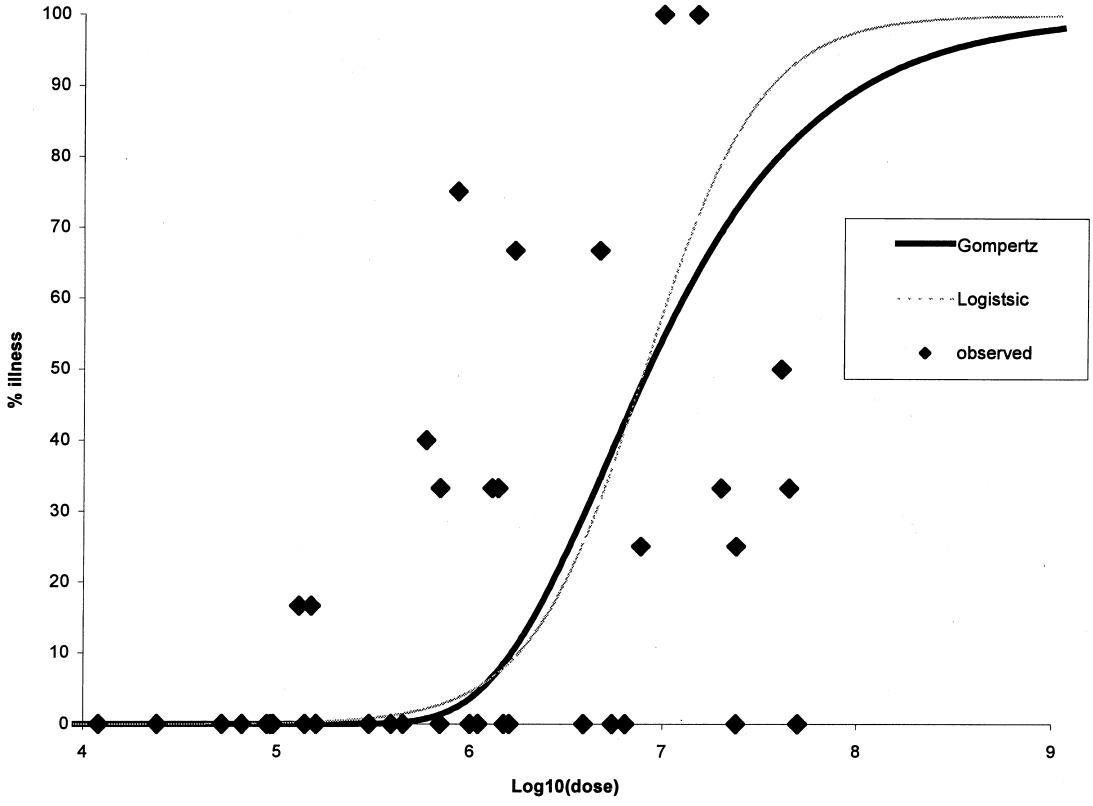

Fig. 3. Human non-typhoid salmonellosis data (McCullough & Eisle, 1951a–d) and models (Coleman, 1998, with permission from *Journal of Food Protection*, copyright held by International Association of Milk, Food, and Environmental Sanitarians).

analysis of variance, both Gompertz and logistic functions are found to provide a statistically good fit to the data (Coleman, 1998) and predict low likelihood of adult illness at challenges of 10^6 non-typhoid *Salmonella* cells for healthy adults. These models (Coleman, 1998) thus include variability due to differences in strain as a parameter with a prescribed distribution.

Serious limitations of the available human data exist, especially since a great proportion of human illness in the US arises from other non-typhoid serotypes, such as *S.* Typhimurium and *S.* Enteritidis (CDC, 1995). Unknown are dose-response effects at low dose exposures of 1, 10, 100, and even 1000 non-typhoid *Salmonella* cells and dose-response effects of more susceptible human sub-populations such as children. Further, emerging pathogens present difficult challenges for dose-response modelers. Emerging pathogens such as *S.* Typhimurium DT104, will require some focused attention by multi-disciplinary risk assessment teams to develop plausible surrogate dose-response models in the absence of direct human data for the particular pathogen strains or serotypes of interest. The need to address surrogate dose-response models mechanistically using in vitro and animal model systems was highlighted by the recently awarded collaborative agreements to extend ongoing human clinical trials by the US Food and Drug Administration under the President's Food Safety Initiative (www.vm.cfsan.fda.gov). The goal of the collaborative research funding is to promote development of plausible dose-response models which are grounded in the biology of pathogenic interactions, utilizing mechanistic approaches to model events of pathogenesis. Accounting for variability in each aspect of the disease triangle (pathogen, host, and environment) is of importance to risk assessors.

Dose-response modelers using data from the human feeding studies generally treat pathogenesis as a black box (Fig. 4, with permission from JFP; Coleman, 1998). The pathogen is administered, and the adverse response, diarrhea, is observed. One might be able to illuminate the black box using more mechanistic approaches (Roth, 1995; Salyers, 1994) such as compartment modeling utilized in pharmacokinetic and pharmacodynamic modeling. Exposure assessment could be extended from farm to stomach, and then dose-response modeling could begin in the lower GI tract on tissue, cellular, molecular, and perhaps genetic levels (Jones, 1997; Neidhardt, 1996; Roth, 1995; Salyers, 1994). However, much research is needed for developing the parameters for such a model in humans.

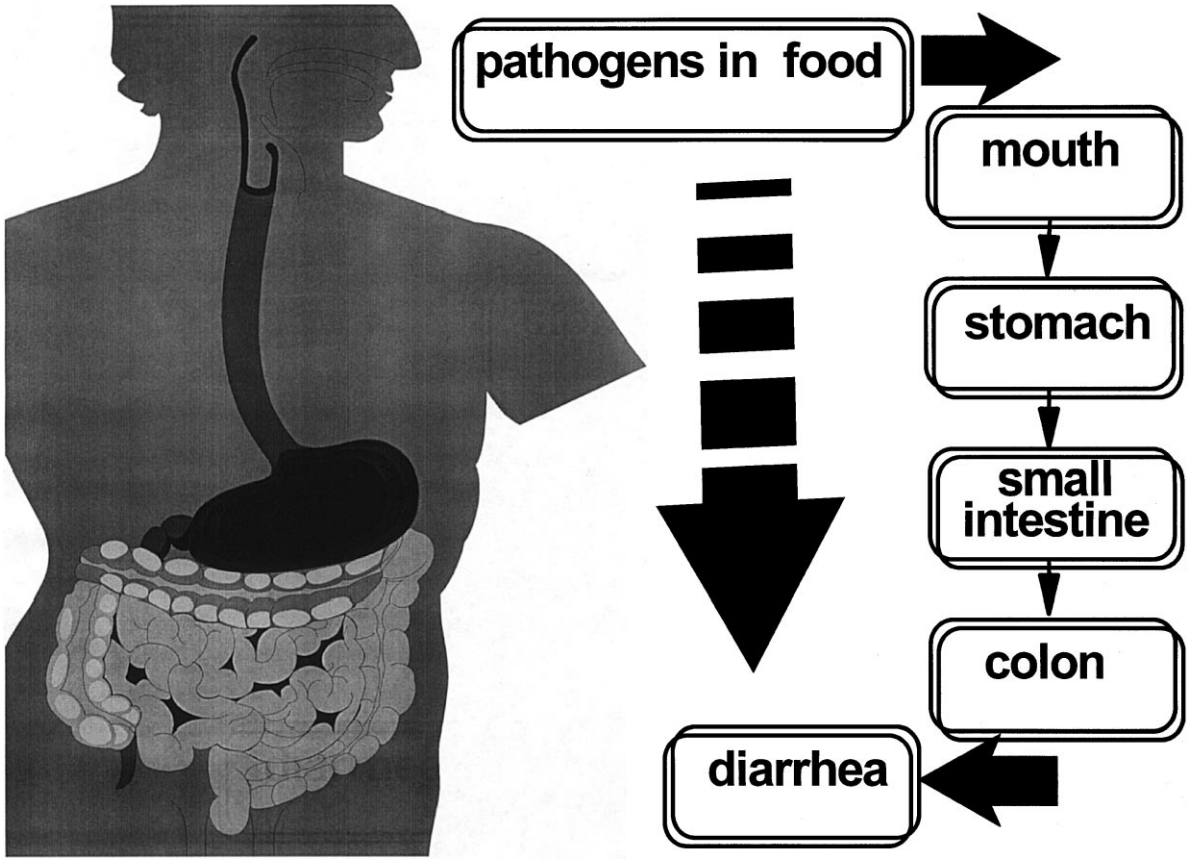

Fig. 4. Black box of pathogenesis (Coleman, 1998, with permission from *Journal of Food Protection*, copyright held by International Association of Milk, Food, and Environmental Sanitarians).

An alternative approach involves consideration of mechanistic data from in vitro and animal models to inform human dose-response modeling. For example, in addition to human data (McCullough, 1951a, 1951b, 1951c, 1951d), murine challenge studies exist that demonstrate differential host susceptibility to non-typhoid salmonellosis (Miller, 1954; Bohnhoff, 1954). Miller (1954) demonstrated that mice treated with streptomycin were much more sensitive to subsequent challenge with *S.* Enteritidis than control mice. The initial murine study was extended as a timecourse experiment in which mice treated with streptomycin were subsequently challenged with *S.* Enteritidis (SE) at 1–5-day intervals following antibiotic administration (Bohnhoff, 1954). The full protective effect of the microflora was nearly restored to control levels by the 5 day interval. However, the mice challenged with SE 1 day following streptomycin disruption of the protective microflora were dramatically more sensitive to challenge. The evidence supporting this conclusion is a 100 000-fold reduction in the ID_{50}, the dose at which 50% of treated animals exhibited infection. The murine data demonstrated dramatic shifts in the shape and position of the murine dose-response curve for salmonellosis relative to time after antibiotic administration, a known risk factor for human gastrointestinal illness (D'Aoust, 1997).

Data from the murine studies can inform and extend the human dose-response model (Fig. 3) to account for variability in host susceptibility. The underlying mechanism of this profound effect seems likely to be a general biological phenomenon, the strong protective effect of the indigenous microflora in healthy animals and greater susceptibility in animals with disruption of their normal flora by antibiotic treatment. A family of murine dose-response models was derived from these studies that reflect differential sensitivity of mice. From our analysis of the murine studies, a family of human dose-response curves (Fig. 5) was generated. The right-most curve in Fig. 5 is the Gompertz curve from Fig. 3, based on the human non-typhoid salmonellosis data of McCullough (1951a,c). The human curve was shifted to the left using a vector from the murine studies for differential susceptibilities 1–5 days after antibiotic administration to the mice. The left-most curve in Fig. 5 represents the most susceptible human, based on pathogen challenge 1 day after antibiotic treatment of mice. As observed in the murine studies, the variability derived for human susceptibility also spans at least five orders of magnitude, the ID_{50} for the most sensitive sub-population depicted in the left-most curve is less than 10^3 bacterial cells versus nearly 10^7 for healthy adults not subject to antibiotic disruption of their indigenous microflora. This family of dose-response curves might be of use in

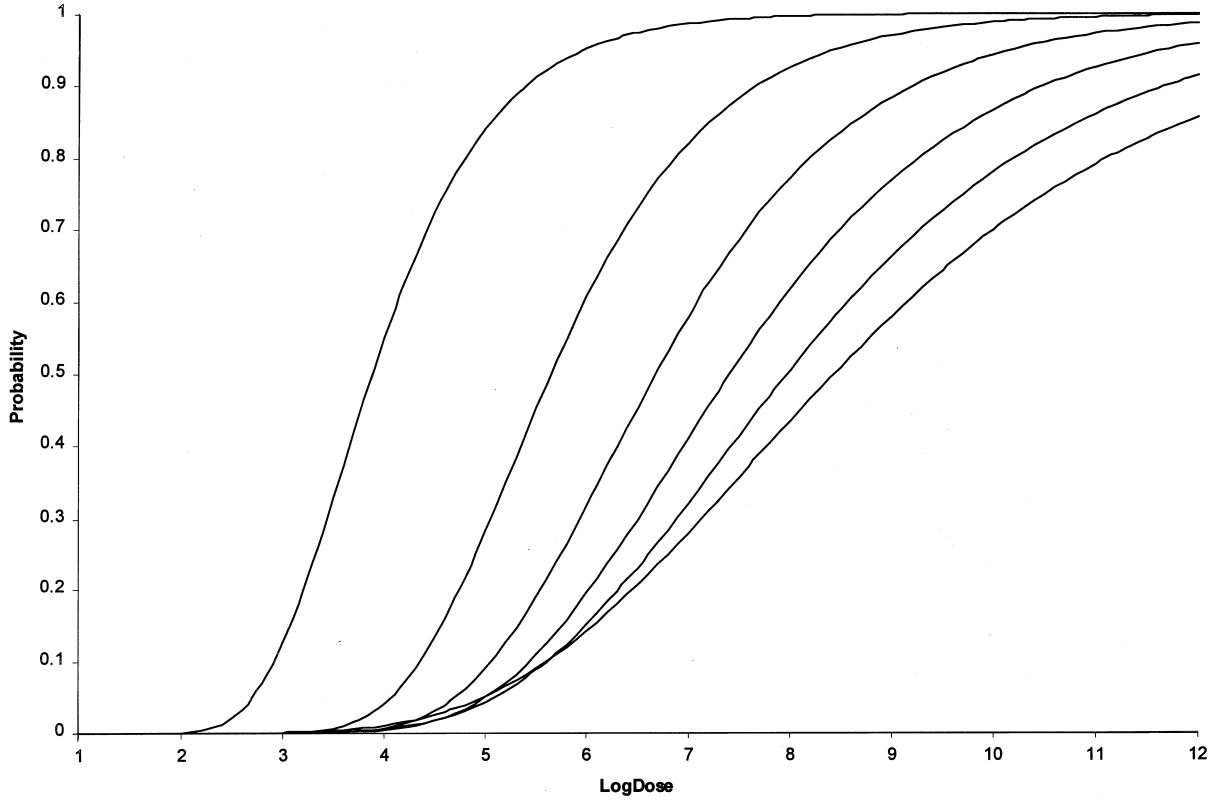

Fig. 5. Family of human dose-response curves informed by mechanistic data from murine studies

future risk assessment for non-typhoid salmonellosis, accounting for the variability in host sensitivity of subpopulations with differential impairment of the full protective effect of the indigenous microflora of a healthy GI tract for adults.

A logical extension of this study is consideration of age-dependent effects associated with enhanced susceptibility of children to gastrointestinal diseases such as non-typhoid salmonellosis (CDC, 1995; Coleman, 1998). Researchers have taken advantage of the observation of enhanced susceptibility in young animals and gnotobiotic animals as sensitive test systems for pathogen challenge studies (Hentges, 1983). Age dependency might be directly explored in mechanistic studies using such animal models or in vitro assays (Roth, 1995; Salyers, 1994). However, in lieu of human data, a risk assessor might assume that the left-most curves derived at 1- and 2-day challenge post-antibiotic administration in mice (Fig. 5) appear to be a more plausible models for the dose-response relationship of children than the right-most curve derived from healthy adult volunteers. These derived 1- or 2-day curves may also be appropriate inferences for children if more frequent antibiotic administration is a factor in their enhanced susceptibility to gastrointestinal diseases. A complete risk assessment should acknowledge the possibility that children may suffer greater frequency of disease as a result of more frequent or higher levels of exposure, due to particular age-dependent behaviors or dietary differences, in addition to likely mechanisms that increase susceptibility.

4. Conclusions

The process of conducting risk assessments is well described as a formal, structured process that is both complex and evolving. Our hope is that our work kindles interest in other organizations to conduct research that is directly applicable to assist risk assessors with the development of more plausible exposure and dose-response models.

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